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PRINCIPAL INVESTIGATOR: Yasmine Kanaan, Ph.D.

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14. ABSTRACT

Genes may play a strong role in prostate cancer etiology but epidemiological studies suggest that prostate cancer risk is largely determined by gene and environmental interactions. In order to explore the effects of UV exposure, serum Vitamin D, and skin color on prostate cancer risk in African American men. Twenty seven affected AA men with histologically diagnosed adenocarcinoma of the prostate; PSA of > 2.5 ng/ml and a positive DRE were recruited under the direction of Dr. Mireku-Boateng from the division of Urology at the Howard University Hospital and nine age and ethnicity matched controls have been recruited through the monthly free screenings program at the Howard University Cancer Center. For each prostate cancer patient and matched control we have collected information on personal and family history, and blood samples for candidate gene testing. In order to measure the intake of dietary Vitamin D each subject completed the standardized food frequency questionnaire and the serum circulating levels of 25-OH Vitamin D have been measured by Enzyme Immunoassay for all participants. To elucidate their exposure to UV from childhood until current the UV exposure questionnaire has been completed. In add

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INTRODUCTION

There are striking differences in the age-adjusted incidence of prostate cancer between different racial groups and between different geographic regions of the world. African American men have the highest incidence of prostate cancer compared to other ethnic groups. This cohort also appears to be present more commonly at an advanced stage with aggressive histology and increased cancer-related mortality. Therefore, there is a critical need to explore the etiologic pathways that contribute to this disparity.

There is accumulating evidence that vitamin D may play an important determinant of occurrence and progression of prostate cancer. Because the prostate cancer mortality rate increases significantly as the availability of UV radiation exposure decreases, and the synthesis of vitamin D depends on UV radiation, it was hypothesized that vitamin D deficiency is a risk factor for prostate cancer (1). Laboratory studies revealed that vitamin D and vitamin D analogues have anti-proliferative and differentiation effects on human prostatic cancer cells *in vitro* (2-7). Clinically, it was claimed that oral administration of 1, 25- dihydroxyvitamin D3, an active form of vitamin D, may delay the recurrence of prostate cancer after primary therapy (8). These documents suggest that vitamin D had a protective effect on prostate cancer

Genes may play a strong role in prostate cancer etiology but epidemiological studies suggest that prostate cancer risk is largely determined by gene and environmental interactions. Increased attention should be placed on research in the African American population on environmental factors such as UV exposure (latitude), lifestyle and diet and their possible interactions with genetic loci.

The goal of this project is to explore the effects of UV exposure, serum Vitamin D, and skin color on prostate cancer risk in a large case-control study of African American men aged $\geq$ 40 years from the Washington, DC area. **Our specific aims are** to (1) recruit 76 prostate cancer cases and 152 age and ethnicity matched controls; (2) assess UV exposure in African Americans prostate cancer patients and matched controls (3) measure modifying factors of UV exposure (skin color, serum 25-OH Vitamin D, and genes involved in Vitamin D metabolism (4) assessment of variation for genes involved in vitamin D metabolism; and (5) determine if UV exposure and modifying factors act alone or interact to affect prostate cancer risk in African Americans.

BODY

Task 1: Start-up phase and clinical database development (1-5 months)

- Develop detailed protocol manual for recruitment and database entry.
- Start advertisements and recruitment process.

Start-up Phase:

At the beginning of the grant period we hired Dr. Desta Beyene and graduate students to support the activities of the grant. The staff became familiarized with the protocol and then received training on the questionnaires to be used in the study including the epidemiologic assessments, family and medical history, socio-demographic information, and Gladys Block Food Frequency Questionnaire. Training on the DermaSpectrophotometer machine for measuring the constitutive skin color was done under the supervision of Dr. Halder in addition to the UV exposure questionnaire.

Ethics training: The students had completed the Howard University Collaborative IRB Training Initiative (CITI) Course and Human Participants Protection Education for Research Teams (online course, sponsored by the National Institute of Health) Course.

Clinical database development:
A database has been developed by the HUCC Biostatistics Core, under the direction of Dr. Kepher Makambi (Director of Biostatistics Core). Joseph Afful, database manager assigned to the project, created a data dictionary and quality control checks for each variable assessed. Quality control checks have been established to ensure the validity of the data ascertained that included the following: variable type, range, list of possible values, internal logical, data completeness and duplicate record checks. The data will be inspected to prevent the entry of duplicate records into the database. Corrections to the data set will be made by a) reselecting the appropriate form, b) querying the data for the record, and c) updating the incorrect information. Estimates of the rate of entry error and the identification of any systematic data entry errors will be found by comparing printouts of entered data to the data encoded on the individual's questionnaire(s). These manual audits may be conducted on a periodic basis. Daily backups will be conducted to protect against accidental corruption or deletion of essential data.

Entering clinical data in database: The data entered into template forms written in Microsoft ACCESS. Each site is kept in duplicate records of all data sent to the coordinating center. The study coordinator monitored the transfer of data and blood specimens in addition to hand checking hard copies before entering the data into the database. All data were entered using the double-keying method. The PI worked with Dr. Lucile-Adams Campbell on aspects of the study dealing with the database and data management.

Recruitment and Advertisement
The Recruitment Core of HUCC is working in support of this prostate project. The responsibility of the Recruitment Core is to coordinate all prostate screening and prostate related activities with the staff of the staff. Meetings continue to occur between the outreach staff and the Recruitment Core in the presence of Drs Kanaan and Adams-Campbell.

Recruitment Protocol and Data Collection
The selection of prospective prostate cancer cases and healthy controls began with the identification of potential participants from a variety of resources available to Dr. Augustine Mireku-Boateng, such as patient files, tumor registry, patient databases, and Cancer Center prostate cancer screenings. Howard University Hospital (HUH) has historically served the African American community in the Washington, DC area. We have recruited 27 affected African American men with histologically diagnosed adenocarcinoma of the prostate; Prostate specific antigen (PSA) of $\geq$ 2.5 ng/ml and a positive digital rectal examinations (DRE) under the direction of Dr. Mireku-Boateng, from the division of Urology at the Howard University Hospital and/or from ongoing HUH/HUCC free prostate cancer screening programs at the Howard University Cancer Center (HUCC). It is important to note that during year 1, we only recruited from one urologic practice. It is our intention to broaden our outreach to include more urologists at HUH and in the community.

Regarding the controls, although we have recruited 9 age-race matched controls. These individuals are healthy unaffected regularly screened volunteers with PSA levels < 2.0 ng/ml, normal DREs and with no history of prostate cancer among first-degree relatives. The controls are recruited from the frequent free prostate cancer screening programs offered at the Howard University Cancer Center and in the community including churches. It is important that we age match all controls with cases. Therefore, we have intentionally delayed our recruitment effort so that we would not have un-matched age controls compared to the cases. However, we have established a recruitment list of men who are interested in participating in the study, are likely controls, and have already undergone DRE and PSA testing during screenings. Thus, we do not anticipate delaying any further and will accrue rapidly using group frequency matching approach whereby we will restrict to ages 40 – 85. Of note, due to the repairs needed on the UV machine, we also could not accrue to the study. It is anticipated that the UV machine will be repaired, however, a back-up machine is needed.

All participants from the study have consented by the Recruiter and completed the questionnaire on demographic and medical history. Each individual was assigned a unique identification number. The numbering system allowed us to track individual samples without the need of personal identifiers (i.e., names and addresses). The numbers were assigned sequentially; Blood were collected from all participants (3 yellow top Vacutainer 6ml tubes for lymphocytes and DNA extractions and 1 red top Vacutainer 6ml tubes for blood chemistry) by certified phlebotomists.

For each prostate cancer patient and control we obtained information on personal and family history, blood samples for candidate gene testing. Each subject answered questions from the UV exposure questionnaire (UVQ) and the standardized food frequency Questionnaire (FFQ) in addition to having their constitutive skin color (M-index measuring using the Dermaspectrophotometer.

Demographic and Medical History:

Prior to the in-person interviews, all subjects signed informed consent. For each prostate cancer patient and matched control we have collected information on personal and family history. Personal history includes ethnicity, alcohol and tobacco intake, occupation exposures, height and weight, medical history and physical activity, and blood samples for candidate gene testing.

We also collected information about household income, home ownership, number of children, and employment of the participant, as well as the highest level of education completed. Collected variables will allow us and other investigators to assess the association between these variables and clinical features.

For the prostate cancer cases, age of onset (mean 64 years, range 47-84), Gleason score (range 4-9) and PSA level (range 8.2-80 ng/ml) were obtained from the cancer registry. Most men are resident in Washington area and the remainder from surrounding Metropolitan areas.

Assessment of UVR exposure:

We have established the protocol for calculating each subject's cumulative sunlight exposure. Each subject answered questions from the UV exposure questionnaire (UVQ). This questionnaire is designed to elucidate their exposure to UV from childhood until current.

Subjects were asked to assess such exposures during age categories; 0-5 years, 6-11 years, 12-17 years, 18-29 years, 30-39 years and 40 years-to age at diagnosis. These data are combined to give total UVR exposure in hr/year.

In the protocol used in this study, each subject's cumulative sunlight exposure will be assessed by a combination of his history of occupational and non-occupational sunlight exposure. All professions were listed and the time periods during which these profession were performed, were recorded.

Food Frequency Questionnaire: Additionally, each subject completed the standardized food frequency questionnaire (FFQ) for dietary assessments. The FFQ is an appropriate epidemiologic method for dietary assessment and is designed to obtain qualitative and descriptive information about usual food consumption patterns. Specifically, the 98.2-item Block Brief 2000 questionnaire will be determined. The Block questionnaire was developed using previously described methods (9), with a food list designed to cover greater than 90% of the average intakes of over 30 nutrients in Whites, African-Americans, and Hispanic Americans. The questionnaire is self administered and shows how often food is consumed as number of times per day, week, months or year. The usual portion size is reflected as small, medium, or large with a picture representation of these sizes. The Block FFQ has been validated and used to assess dietary intake in an African American population (10). The completed FFQs will be sent to the Block Dietary Data Systems in Berkely, CA for component analysis.

Assessment of Skin Color: Measurement of skin pigmentation was carried out using the computerized narrow-band reflectometer called the Derma Spectrophotometer (Minolta Chromameter, Courage and Khazaka Mercameter). Using two wavelengths, the instrument records the reflectance of light emitted on the skin. The results are expressed in terms of erthema (E) and melanin (M) indices (0 to 100%). The inner arm is used to measure constitutive skin pigmentation. Measurements on the forehead and back of hand (facultative skin pigmentation) were taken for the exposed skin area. Three separate measurements of E and M are taken at all three sites and the average M value used in the analysis. The difference between constitutive (inner arm M) and facultative (forehead M) has been proposed as a quantitative index of sun exposure that is related to cumulative lifetime sun exposure (tanning potential).

Task 2: Data collection (Months 3-24).

- Extraction of genomic DNA from blood samples.
- Run serum 25-OH Vitamin D assays.
- Enter clinical data in database.

DNA Extraction:

After the interview, blood was drawn from consenting subjects. The lymphocytes were separated from the blood of patients and controls by using Lymphoprep solution (Axis-Shield POCAS, Oslo, Norway). The solution contains 9.1% (w/v) sodium diatrizoate and 5.7% (w/v) polysaccharide. It is a one-step centrifugal technique for isolation of lymphocytes.
The Buffy-coat leukocyte fraction will be used as the source of genomic DNA. Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Maxi isolation kit as described

by the manufacturer (QIAGEN Inc.). The procedure involved cell lysis, proteinase K-treatment, protein precipitation and DNA precipitation. The DNA concentration was determined by the Nano-Drop (ND-1000). The collected Blood and DNA from Subjects is stored in a locked freezer which is located in a secured area at Howard University Cancer Center and an identification code were used on blood samples.

Serum 25-OH Vitamin D assays:

We have established and standardized the conditions in our laboratory the assay for the quantitative determination of the 25-OH Vitamin D in serum by using the Enzyme-based-Protein-Binding-Assay from ALPCO (Windham, NH). Photometric measurements of the standard concentrations (0, 6.4, 16, 40, 100 and 200 nmol/L) were used to establish a standard curve using a four-parameter model. The equation used was $Y= (a-d)/ (1+(x/c)^{b}) +d$, where y= absorbance reading @450nm; a= 1.20958, b=0.71301, c=19.72576, d=0.06209 and x= concentration in nmol/L. The actual Vitamin D concentration will be calculated based on the relationship of 1ng/ml = 2.5 nmol/L

This dose response curve of the absorbance unit vs. concentration is generated using the results obtained from the calibrators. Concentrations of 25-OH Vitamin D, present in the subjects' samples, will be determined directly from this curve.

Denaturing High-Performance Liquid Chromatography (DHPLC):

DHPLC analysis is a chromatographic mutation analysis method that relies on the formation and separation of double-stranded DNA fragments that contain mismatched pairs from a pool of PCR amplified DNA fragments known as heteroduplex DNA. DHPLC is based upon heteroduplex detection and the heteroduplex profiles are identified by visual inspection of the chromatograms on the basis of the appearance of additional earlier eluting peaks. Corresponding homozygous profiles show only one peak. Analysis is carried out on an automated DHPLC instrumentation equipped with a DNASep column (Transgenomic Inc., San Jose, CA). Samples will be separated (flow rate 0.9 ml/min) through a linear acetonitrile gradient (Fisher, Chicago) and detected by online ultraviolet (UV) absorbance monitoring at 254 nm.

We have already standardized the polymerase chain reaction (PCR) and DHPLC conditions for analyzing the vitamin D receptor (VDR) gene. Polymerase chain reactions (PCR) of VDR exons and intron-exon boundaries was performed in a 25 ul volume (containing 40 ng of genomic DNA as a template, 0.4 uM each of exon-specific forward and reverse primers , 1X Gold Buffer, 0.2 mM dNTP mix; 2 mM $MgCl_2$ and 1 U AmpliTaq Gold polymerase . PCR was performed in an AmpGene 9700 thermal cycler (Perkin-Elmer 600, Foster City, CA) as follows: initial denaturation at 95 ^{0}C for 3 min to activate the enzyme; 5 cycles of 30 sec at 95 ^{0}C, 40 sec annealing at 65 ^{0}C (decrement of 2 ^{0}C per cycle), and 1 min extension at 72 ^{0}C followed by 30 cycles of 30 sec at 95 ^{0} C, 40 sec at 55^0 C, and 1 min at 72^0 C; and final extension at 72^0 C for 7 min.. Crude PCR products were checked by agarose gel electrophoresis before DHPLC analysis, to make sure that no additional bands occurred that could lead to artificial heteroduplex conformation. The PCR products were subjected to an additional 10 minutes at 95^0 C denaturing step and then left at room temperature for 10 minutes for reannealing prior to analysis. The start- and end-points of the gradient were adjusted according to the size of the PCR products using an algorithm provided by the WAVE maker system control software. The temperature required for the optimum resolution of heteroduplex molecules is determined by use of the DHPLC melting

algorithm and pre-testing of several temperatures. The samples that show heteroduplex peaks will be sequenced in both directions on an ABI 377 automated sequencer using the fluorescent labeled Big-dye terminator cycle sequencing kit (ABI) or ET terminator cycle sequencing kit (Amersham).

KEY RESEARCH ACCOMPLISHMENTS:

- Established Recruitment Infrastructure
- Created Database
- Trained Staff
- Started Recruitment of Cases and Controls
- Collected Lymphocytes, serum and DNA from subjects have been isolated and stored in a locked freezer which is located in a secured area at Howard University Cancer Center and an identification code were used.
- Established and standardized the conditions in our laboratory the assay for the quantitative determination of the 25-OH Vitamin D in serum by using the Enzyme-based-Protein-Binding-Assay from ALPCO.
  PCR and DHPLC conditions for detecting variants have been established for VDR gene.

REPORTABLE OUTCOMES: Research and laboratory technology training:
The training was for graduate students: Altreisha Foster and Douglas White (Microbiology Department), Hilaire kenguele (Biology Department) and undergraduate student: Vonetta Williams (Chemistry Department), on the use of technology and software for primers designing, DNA extraction, and ethics training.
Ethics Training: The students had completed the Howard University Collaborative IRB Training Initiative (CITI) Course and Human Participants Protection Education for Research Teams (online course, sponsored by the National Institute of Health) Course.

POTENTIAL PROBLEM: The DermaSpectrophotometer used to measure the skin pigmentation under the supervision of Dr. Halder, Chair of Department of Dermatology at the Howard University Hospital. This machine is the only one in the hospital and recently the machine is broken and sent for maintenance. Therefore, we need to purchase a new machine in order to continue measuring the skin pigmentation.

CONCLUSIONS: Prostate cancer is a complex disease with both genetic and environmental components. Epidemiological data reveal that African American men have the highest incidence and mortality rates for prostate cancer. Despite its high prevalence among African Americans, very little is known regarding genetic predisposition and environmental influences on prostate cancer. We are particularly intrigued by the interaction of UV exposure and modifiers of vitamin D level in the serum (skin color, genes and diet). This observation leads to the hypothesis that the higher incidence of prostate cancer in elderly men and black men may be related to vitamin D exposure, which is decreased with aging skin and darker skin

pigmentation. Our working hypothesis poses that increased incidence of prostate cancer and mortality in African Americans involves a dynamic interplay of environmental factors such as diet and UV exposure in addition to genetic factors, some which directly influence serum vitamin D levels. Our work thus is extremely promising. Once completely analyzed, our data will contribute to the current knowledge on DNA sequence variations. But more importantly, analyses of our populations will allow us to dissect the role DNA sequence variations play in prostate carcinogenesis, response to treatment and disease aggressiveness in high risk populations.

FUTURE DIRECTIONS:

- Will continue recruiting Subjects from the Washington, DC area either from the urologic practice Augustine Mireku-Boateng, MD, and other urologists at Howard University Hospital.

- For each prostate cancer patient and matched control we will obtain information on personal and family history, and collect blood samples for candidate gene testing and serum vitamin D level. Personal history includes ethnicity, alcohol and tobacco intake, occupation, and medical history. Each subject will answer questions from the UV exposure questionnaire (UVQ), standardized food frequency questionnaire (FFQ) and their constitutive skin color will be measured using the Derma spectrophotometer.

- We will build a comprehensive data resource to explore the interactions of Vitamin D levels, UVR exposure, genes and diet in African American men with and without prostate cancer.

- Statistical analysis for each study aim is planned to exploit univariate and multiple logistic regression models.

- Dr. Kanaan and Dr. Lucile-Adams Campbell will meet regularly with the research team to discuss the outcome and the project progress.

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APPENDICES:

1. Howard University IRB renewing approval letter for the Year 2006-2007.
2. Howard University IRB annual status report form.
3. Consent form and flyer.

HOWARD UNIVERSITY

June 20, 2006

OFFICE OF THE PROVOST
INSTITUTIONAL REVIEW BOARD

Yasmine M. Kanaan, Ph.D.
Cancer Center
Department of Medicine
College of Medicine
Howard University
Washington, DC 20059

RE: **IRB-02-MED-42** "UV Exposure, Vitamin D, and Prostate Cancer Risks in African Americans."

Dear Doctor Kanaan:

The Institutional Review Board (IRB) acknowledged receipt of the request to continue the above-referenced protocol. It was noted that 36 participants were enrolled during the last approval cycle. It was approved at the June 7, 2006 meeting and will expire June 30, 2007, in keeping with the stated project period. The HU IRB Federal Wide Assurance number is FWA00000891.

Please be advised that in accordance with Federal and University policies, all informed consent documents are to be kept on record with this project and should be archived at least three (3) years after the date of the last IRB approval. The enclosed IRB date-stamped consent form should be used when obtaining informed consent. All other versions of the consent form and flyer should be destroyed. In the event that any changes are made in the protocol, including personnel changes, they are to be approved by the Board prior to their initiation. Finally, certification of the completion of the required educational program by all personnel on this protocol should be forwarded to the IRB. Information concerning this requirement can be found at www.huirb.howard.edu

Annex II, Room 214
Washington, DC 20059

(202) 806-7812
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Should you anticipate renewing this protocol annually, a status report is to be submitted to the Board **90 days prior to the expiration date.** If not, a close-out report is to be submitted to the Board **within 90 days after the completion of this study.** The Status Report Form can be downloaded from the HUIRB web site.

During the project period of this research, you may be monitored by a site visit team from the IRB. You will be notified in advance if your project is chosen for the on-site monitoring.

Thank you for keeping the Board apprised. We wish you continued success in your research endeavors.

Sincerely,

Warren K. Ashe, Ph.D.
Acting Chairman

cc: Robert E. Taylor, M.D., Ph.D., Interim Dean, College of Medicine
Duane T. Smoot, M.D., Chairman, Department of Medicine
Lucile L. Adams-Campbell, Ph.D., Director, Cancer Center

Enclosures
WKA/dkc

HOWARD UNIVERSITY CANCER CENTER
2041 Georgia Avenue, N.W.
Washington, D.C. 20060

Consent to Participate in a Genetic Research Study

Subject ID: _____

Project Title: UV Exposure, Vitamin D, and Prostate Cancer Risk in African Americans

Investigator: Yasmine Kanaan, Ph.D.

Introduction

Dr. Yasmine Kanaan of Howard University Cancer Center is involved in a study to identify risk factors involved in prostate cancer. We invite you to take part in this research study. It is important that you read and understand several rules that apply to everyone who participates in our studies: (a) taking part in this study is entirely voluntary; (b) personal benefits may not result from taking part in the study, but knowledge may be gained that will benefit others, and (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. Feel free to ask any questions you have about this study with the staff members who explain it to you.

Explanation of the Subject

Purpose: In the United States, prostate cancer is the most common cancer in men. African American men have the highest rate of prostate cancer in the world. Also, more African American men die from this disease than any other men in the world. Doctors may be able to decrease prostate cancer in African American men and those dying from the disease if research scientists can identify risk factors before it get out of control. If these risk factors are found, it may be possible to identify persons who are at greater risk of developing prostate cancer. Such persons can then be followed very closely by their doctor for early detection of abnormal changes and treated before cancer develops. Early detection and treatment can prevent the

development of cancer. This study will determine if exposure to ultraviolet light, and changes in vitamin D (normally contained in your diet) levels and defects in the genes which control vitamin D utilization in the body might be important risk factors in the development of prostate cancer. You have been selected to participate in this research study because you are an African American male, over 40 years old, and diagnosed with prostate cancer. Or a man without prostate cancer, no family history of prostate cancer among first-degree relatives and prostate specific antigen level is less than 2.5 ng/ml and a negative digital rectal exam.
The following tests or procedures are needed for the project.

Tests or Procedures to be Performed

As a part of your participation in this project you will be interviewed by a trained interviewer who will ask you questions about your medical condition(s) and your family history. There will only be a single 1 hour visit needed in order to complete four questionnaires which include demographic (such as name, address, age , etc.), sunlight exposure, and dietary history (certain kinds of food intake) , and a blood draw. You will than have your skin color measured with a colorimeter, an instrument that shines a bright light on the skin surface and measures light which is reflected. Skin color is measured in two places -on the inside of the upper arms and on the forehead. Once this process is complete, your participation in the study will have ended. The interview, exam and blood collection will occur in the urology clinic of the Howard University Hospital. We need a blood sample in order to look for specific information about your gene(s), the potential risks and benefits if you decided to participate in the study will be discussed with you and a blood sample of about 40cc (almost 3 tablespoons) will be drawn from your arm. The genetic material (DNA) from cells in your blood will be examined. All procedures for this study will be done at no cost to you. We expect to enroll 76 prostate cancer cases and 152 controls (men without prostate cancer), all African American men like yourself, and over 40 years old. You have been selected into one of two groups either because you have a positive prostate cancer test or you will serve as a healthy control (no prostate cancer). If you have prostate cancer, your clinical and pathology records will be accessed as part of your participation in the study. Your participation may be terminated at any time by the investigators if it is determined that you reported false information about yourself and family history.

Participant initials_____ Witness initials_____

Risks/Discomforts: You may experience some discomfort from the needle prick in your arm and there is a chance that you may bruise due to a blood leaking under the skin. This is no reason for alarm. The bruise should clear up on its own in a day or two. There is a very small chance you may feel a little light-headed while the blood is being drawn. Talking about family health problems may be difficult for you, and family relationships may be affected. We will not release any information about you to any third party without your written permission. This includes your insurance companies and employer.

Sample collection/storage: The principal investigator will arrange to have a blood sample collected from you. DNA collected from your blood sample will be stored in locked freezers contained in a secured building on the Howard University campus. Samples collected will be stored for the period of this study and then destroyed by burning. Controls to protect your identity have been put in place. No one other than those who enroll you in this study will know your name and address. This information will remain secured in a locked file at Howard University. To prevent others from knowing your name, an identification code will be used on blood samples and all information on you. You have the right to withdraw from this study any time. Any samples you have contributed will be discarded at the point of your withdrawal. Results obtained prior to your withdrawal from the study will be maintained, but your privacy will be preserved in any specific publication of data by only reported grouped data. Your withdrawal from the study will in no way affect access to medical care at Howard University Hospital for which you are otherwise eligible.

During this study, you will be asked to provide blood. These samples will be used for determine if exposure to ultraviolet light, and changes in vitamin D (normally contained in your diet) levels and defects in the genes which control vitamin D utilization in the body might be important risk factors in the development of prostate cancer, and may also be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. Should your donated samples lead to the development of a commercial product, Howard University will own it and may take action to patent and license the product. Howard University does not attend to provide you with any compensation for your participation in this study nor for any future value

Participant initials_____ Witness initials_____

that the samples you given may be found to have. You will not receive any notice of future use of your samples.

USE OF BLOOD FOR FUTURE RESEARCH STUDIES

I agree to the storage and use of my excess blood for future Howard University Institutional Review Board (IRB) approved cancer research studies.

YES ☐ NO ☐ Patients Initials_____

The responsibility of Howard University Institutional Review Board (IRB) is to ensure that the protocol is in compliance with applicable human subjects' protection regulations and ethical guidelines and to protect human subjects in research.

Benefits: There may be no direct benefit to you, but you may experience positive feelings simply from contributing to the effort to better understand this disease that has affected you if diagnosed with prostate cancer or if you participate in this study as a control (no prostate cancer). Identification of optimum sunlight exposure, vitamin D levels and/or changes in genes may provide additional knowledge of how the disease develops and eventually lead to better ways of diagnosing and treating the disease. Your results will not be made known to you because we may not have enough information to accurately interpret the results derived from this study at the present time.

Compensation: You will be paid by researchers $25 for your participation unless you withdraw before the completion of the exam and interview.

Confidentiality: When results such as this are reported in medical journals or at medical meetings, the identification of those taking part is withheld. Records from this research study will be kept in a separate set of files and locked when not being used by project staff. The principal investigator, the Howard University Institutional Review Board, and the U.S. Army Medical Research and Materiel Command will have access to the records.

Representatives of the U.S. Army Medical Research and Materiel Command, which is funding this research, are eligible to review research records as part of their responsibility to protect human subjects in research.

HOWARD UNIVERSITY
IRB
APPROVAL PERIOD
JUN 3 0 2007

Participant initials_____ Witness initials_____

Injuries: Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study.

Problem or Questions: Should any problems or questions arise with regard to this study, your rights as a participant in clinical research, you decide to withdraw from the study, or any research-related injury, you should contact the principal investigator, Yasmine Kanaan, Ph.D. Telephone: (202) 806-9540 or Dr. Lucile Adams-Campbell, Telephone: (202) 806-7697. If there are any questions that you would like to discuss with someone other than the investigators on this project, please feel free to call the Office of the Executive Secretary, Howard University Institutional Review Board at (202) 806-7818.

Consent Document: It is suggested that you thoroughly read and keep a copy of this document for your later reference and personal records.

PLEASE COMPLETE ITEM BELOW:

I have read the explanation about this study and have been given the opportunity to talk about it and ask questions. I hereby consent to take part in this study.

Participant's Name (please print)	Date	Participant's Signature	Date

Permanent Address

Witness's Name	Date	Witness's Signature	Date

Investigator's Signature	Date	Investigator's Signature	Date

HOWARD UNIVERSITY
IRB
APPROVAL PERIOD
JUN 30 2007

Participant initials_____ Witness initials_____

Researchers at Howard University Cancer are seeking African American Men to participate in a research project "UV Exposure, Vitamin D, and Prostate Cancer Risk"

The goal of this project is to determine if exposure to ultraviolet light (from sunlight), and some changes in vitamin D (normally contained in your diet) levels and changes in your genes might be important risk factors in the development of prostate cancer.

The eligibility criteria:

- 40 years and older African American male
- diagnosed with prostate cancer
- Or without prostate cancer and no history of prostate cancer

Time: 1 hour study visit

Procedure:

- Questionnaires: demographic (such as name, address, age, etc.), sunlight exposure, and dietary history (certain kinds of food intake) and family history of cancer.
- A blood sample of about 40cc (almost 3 tablespoons) will be drawn from your arm.
- Doctors and pathology records will be accessed as part of your participation in the study.
- Measure your skin color with an instrument that shines a bright light on the skin surface.

Cost: All procedures for this study will be done at no cost to you.

Compensation: You will be compensated for your participation.

For more information please contact (202) 806-5640 OR (202) 806-9540

APPENDIX F

ANNUAL STATUS REPORT FORM
FOR PROJECT INVOLVING HUMAN PARTICIPANTS

Reporting Period: From: July 1, 2005_____ To: _May 30, 2006_____

IRB Number: IRB-02-MED-42._____

1. Principal Investigator's Name, Department, Telephone Number and E-Mail Address: Yasmine Kanaan, Ph. D. Microbiology Dept. 202 806 9540, ymkanaan@howard.edu_____
2. Title of Project:__ "UV exposure, vitamin D, and prostate cancer risks in African Americans"_
_____
3. What is the source of the funds to support this project?__DOD_____
4. State the Project Period: From _2005_____ To_2008_____
5. If the project is not yet active, please indicate a potential start date: _____
6. Give the anticipated end date of the study: June 30, 2008_____
7. Does this project involve drugs? Yes ____ No _X___ If your response is "yes," has it been reviewed by the Pharmacy & Therapeutics Committee (PTC) of the Howard University Hospital?
Yes ____ No ____ If your response is "no," two (2) copies of the IRB approved protocol must be forwarded for PTC review in care of the Director of the Pharmacy, Room BB10, Howard University Hospital.
8. State the number of participants enrolled this reporting period: _36_____. This applies to all participants in the project, including normal (volunteers), controls and experimental participants.
9. State the number of currently enrolled participants that are to be followed. __?_____.
10. State the total number of informed consent forms obtained since the beginning of this project and indicate where they are located and under what conditions they are maintained. _36 informed consent forms obtained and all are locked in a closed file cabinet in the PI's office (Rm 420/ HUCancer Center)_____
11. State the number of banked tissues/DNA sets/blood samples being stored: _DNA_36 samples_____
11a. Indicate where they are located and under what conditions they are maintained: _stored in -80^{0} C freezer, Lab 416/ Cancer Center_____
11b. Indicate whether they are being shared and with whom they are being shared: _Non_____
_____
11c. State the number of lost or destroyed banked tissues/DNA sets/blood samples: __Non_____
Explain the circumstances: _____
12. Have any adverse reactions or deaths occurred during this reporting period? Yes ____ No __X__ If your response is "yes," please describe each event in detail using continuation pages which should be attached to this form. Include a discussion of the corrective measures taken to prevent each recurrence and whether they were previously reported. PLEASE NOTE: All adverse reactions and deaths must be reported immediately.
13. Describe any major protocol or personnel changes made during this reporting period using continuation pages, if necessary. Biographical sketches should be attached for all new personnel.
_____
14. Attach a copy of an executed informed consent form that was obtained during this reporting period. If this protocol is on-going, attach a clean consent form for the next requested approval period.